A RECONNAISSANCE OF THE RELATION BETWEEN DESICCATION AND CARBON DIOXIDE PRODUCTION IN ANIMALS.

G. T. CALDWELL,

HULL ZOÖLOGICAL LABORATORY, THE UNIVERSITY OF CHICAGO AND THE BIOLOGICAL LABORATORY OF THE UNIVERSITY OF ARIZONA.

I. INTRODUCTION.

A fairly comprehensive review of the literature on the desiccation of animals reveals work largely of two general types, (1) the effects of desiccation on the behavior of the animal as shown by Tower (1906), Hennings (1907), Jacobs (1909), Breitenbecker (1911), Shelford (1913, 1914a), Chenoweth (1917), Wesse (1917), Hamilton (1917), and Bodine (1923), and (2) the water content and the vital limits of desiccation of animals as determined by Leeuwenhoek (1701), Baker (1764), Doyére (1842), Semper (1881), Durig (1901), Babcock (1912), Schmidt (1918), Bodine (1921), and Hall (1922). From the standpoint of possible physiological changes resulting from water losses in animals, little has been done and it is the purpose of this paper to point out certain definite relations between desiccation and the physiological states of a limited number of species, as determined by the excretion of carbon dioxide.

The work has been made possible largely through the kind suggestions and helpful comments of Dr. W. C. Allee. Thanks are also due Dr. L. H. Hyman for assistance in the collection and identification of material.

II. MATERIAL.

Many animals were tried and rejected as unsuitable because of the inability to keep them quiet throughout the determinations. The material was selected so far as possible with regard to the natural habitat of the animals. The meal worm, larva of *Tenebrio molitor* Linn., a xerophilous animal, the larva of the eight-spotted forester moth, *Alypia octomaculata* Fabricius, a mesohygrophilous animal, and the slug, *Agriolimax campestris*

Binney, the slimy salamander, *Plethodon glutinosus* Green, the cricket frog, *Acris gryllus* Le Conte, all hygrophilous animals. The natural environments of these animals give a fairly wide range of maximum evaporation. With the exception of *Acris* and *Tenebrio*, the animals were kept in the laboratory only a few days and in all cases were under as nearly natural conditions as possible.

III. Methods of Desiccation and Carbon Dioxide Determination.

The desiccating apparatus consisted of a series of washing bottles so arranged that air could be drawn through sulphuric acid, granulated zinc, and glass wool into the desiccating chamber. The air from the desiccator was bubbled through a methyl orange solution for three hours to test for sulphuric acid. This test was repeated at intervals throughout the work and at no time were there indications of sulphuric acid in the air.

The respiratory apparatus devised by Osterhout was used to make the carbon dioxide determinations. Since this apparatus has already been described (Osterhout, 1918), a brief summary will suffice: the apparatus consists of a closed system in which air is circulated by means of a pump. There are two possible ways for the air to pass from the animal chamber, either directly into a Pyrex glass tube containing an indicator solution, or indirectly through a container of sodium hydroxide which removes the carbon dioxide from the air before it reaches the indicator solution. Thus, the desired color may be restored to the latter without the necessity of a change of indicator. This is highly advantageous because a series of determinations is possible without the admission of air to the closed system. In all experiments, 4 cc. of an aqueous solution of phenolsulfonephthalein were used. The indicator solution was made at the beginning of each experiment in sufficient quantity to last throughout one complete experiment, except when the experiment ran for several days, which occurred only in the work on the larvæ of *Tenebrio* and *Alypia*. A north skylight lamp and a white background made of absorbent cotton were employed to avoid the changeable color quality of daylight and furnish a constant light for work at night upon the

critical comparisons of the known and unknown indicator
solutions.

Wherever the respiration time of animals appears in this
paper, it is to be taken as the time in seconds required by the
animal, or animals, to excrete sufficient carbon dioxide to change
4 cc. of standard indicator solution from pH 7.8 to pH 7.2. No
attempt was made to determine the actual quantities of carbon
dioxide produced by the animals, since all comparisons were made
on the basis of time required for the excretion of sufficient carbon
dioxide to produce this standard change in pH value.

The control of the movements of the various animals in the
respiratory chamber was on the basis of tropistic responses to
light or contact. In no instance was cessation of activity
produced through mechanical means, and no experiments have
been recorded in this paper where the animal was moving when
the carbon dioxide determinations were made. Movements
could easily be detected through marked irregularities in respi-
ration time.

Controls were run on all experiments. The control animals
were kept under the same conditions as the experimental animals
except they were not desiccated and exclusive of one control in the
experiments on *Tenebrio*, neither controls nor experimental
animals were fed during the experiment.

The animals were weighed in previously adjusted weighing
bottles immediately after the carbon dioxide determinations were
made. This proved preferable to weighing before the determi-
nations were made since it reduced the stimulating effect of
handling and consequently the animals came to rest in the
respiratory apparatus more quickly. The animals undoubtedly
lost weight in the animal chamber of the respiratory apparatus in
some instances and gained weight in other instances, depending
upon the state of desiccation, but the gain or loss in the respiratory
chamber was so slight compared with the loss in the desiccator
that it was disregarded. All weights are given in milligrams as a
matter of convenience.

The general procedure was the same for all of the experiments
conducted. The normal respiration time of the experimental
animal was determined; three consecutive readings were averaged
for each determination. These readings varied somewhat

because of the impossibility to detect always the exact end-points; in some instances the variation was as much as 2 per cent and throughout the work the variation was greater with the shorter respiration periods than with the longer. The animal was then weighed and placed immediately into the respiration chamber of the desiccator. The normal respiration time of the control was determined and its weight recorded while the experimental animal was being desiccated. This was repeated at intervals, usually until the experimental animal showed no signs of life. The intention was to obtain the rates of carbon dioxide excretion at approximate stages of from 5 to 10 per cent. loss in body weight. The time necessary for a loss in body weight of about 10 per cent. was previously determined with animals of similar weight.

IV. Experimental Results.[1]

A. Xerophilous Animals.

Meal Worm, Larva of Tenebrio molitor Linn.—These worms were kept in the laboratory in open Mason jars partially filled with air dried wheat bran. Five series of experiments were performed. Three worms were used in each experiment to reduce the respiration time. The controls of four of the five series were not fed. The controls of the fifth series were fed throughout the experiment. The first readings were the most difficult to obtain on account of the activity of the animals. A dark chamber was the only means used to keep the animals quiet, although it was found that animals placed with their bodies in contact with both the bottom and side of the animal chamber not only remained quiet but also were less inclined to aggregate. After 10 hours in the desiccator the meal worms were inactive when undisturbed. In the first four series, carbon dioxide determinations were made regularly at 24-hour intervals for six days. In the fifth series determinations were made at shorter intervals. Evaporation from the meal worm is very slow even in dry air. Hall (1922) found that it required 1,084 hours to desiccate a meal worm to 52.6 per cent. of its body weight. While this loss was a greater percentage of body weight than the average percentage of water contained in the body, the worm lived two days after desiccation.

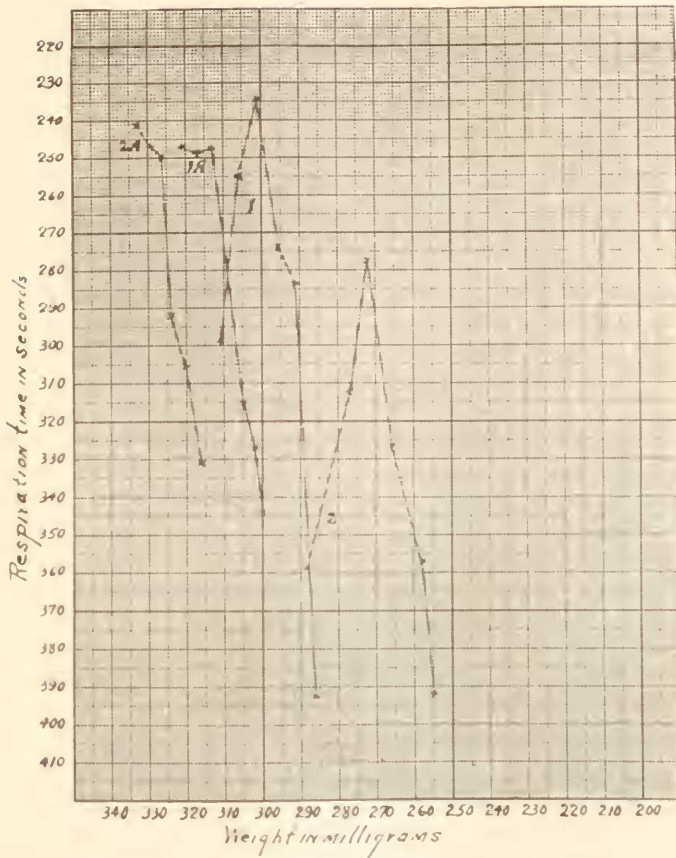[1] Complete experimental data are on file in the University of Chicago Libraries.

FIG. 1. The effect of desiccation on the carbon dioxide production of the meal worm. Points (x) denote determinations at 24-hour intervals. Curves 1A and 2A, controls of 1 and 2 respectively. Note marked decrease in carbon dioxide output at 72-hour determinations of both experimental animals and controls.

B. *Mesohygrophilous Animals.*

Larva of the Eight-spotted Forester Moth, Alypia octomaculata Fabricius. — This larva was taken from the Boston ivy (*Ampelopsis*), which grew on the walls of the laboratory, and placed directly into the animal chamber of the respiratory apparatus. Preliminary experiments proved the animal to be very active and stimulated by contact with the glass wall of the chamber. Fairly satisfactory results were obtained by placing a rough piece of wood, which had been oven dried and tested for carbon dioxide, on the bottom of the chamber upon which the animal became

quiet when the chamber was darkened. This piece of wood was
kept in the animal chamber throughout the experiment in order
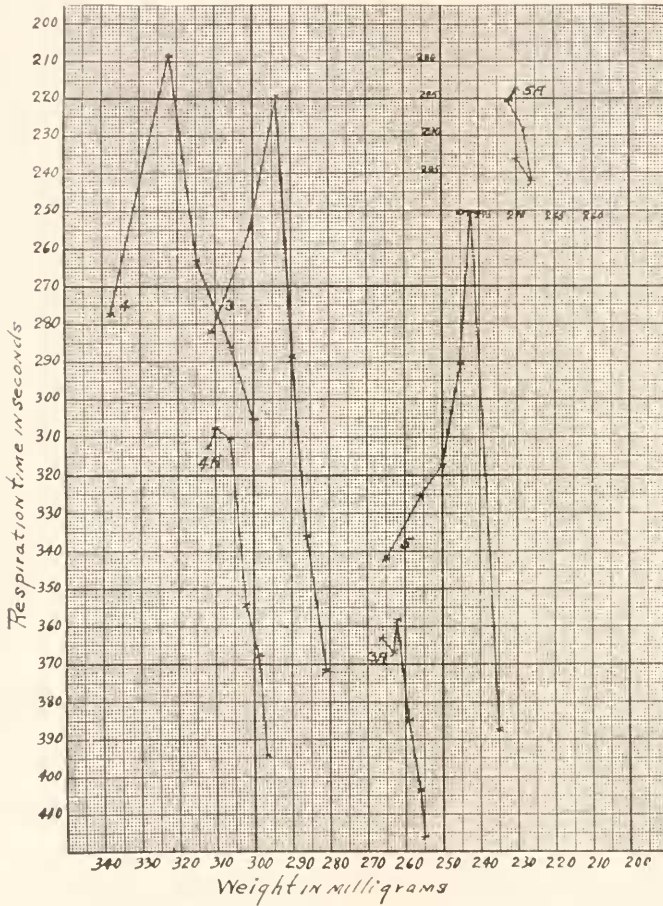that the reduction in the volume of the apparatus would remain
constant.

FIG. 2. The effect of desiccation on the carbon dioxide production of the meal
worm. Experiments 3 and 4 with controls 3A and 4A on animals not fed during
experiments and with determinations at 24-hour intervals. In experiment 5 the
control animals, curve 5A (twice experimental scale) were fed. Point (o–x), curve
5, denotes carbon dioxide determination after 68 hours of desiccation.

Both the experimental animal and control lost about 55 per
cent. of the body weight in 70 hours. The animals were still
active but much reduced in size. The experimental animal and

control showed a decrease in the rate of carbon dioxide production, the experimental animal from the first desiccation, and the control after 8 hours.   The available data are insufficient to permit of any definite conclusions.   However, two possibilities

Fig. 3.   The effect of desiccation on the carbon dioxide production of the larva of the eight-spotted forester moth.   The time intervals of determinations (x) were the same for the experiment, curve 1, and control, curve 1A; first, 3 hours, second, 5 hours, third 24 hours, fourth, 18 hours and fifth 14 hours.

are suggested by this and several preliminary experiments: one, the partial closure of the spiracles to retard the loss of water, and the other, the effects of starvation.   Similar results were obtained in trial experiments with the larvæ of two other species of Lepidoptera (unidentified) and the centipede, *Scolopendra heros*.

C. *Hygrophilous Animals.*

Slug, Agriolimax campestris Binney.—The experimental animals were taken from their normal environment in a greenhouse and placed in the animal chamber of the respiratory apparatus. The control animals were kept in moist filter paper in a closed dark chamber where the rate of evaporation and temperature were approximately that of the normal environment.   Single individuals were used in each of the four series of experiments recorded.   The animal chamber of the respiratory apparatus was

covered with black paper and a small opening at one side per-
mitted of observation without disturbing the animal by the
movements involved in the manipulation of the apparatus. The
slugs usually came to rest in a short time in the darker region of
the chamber. Series of determinations were made until the
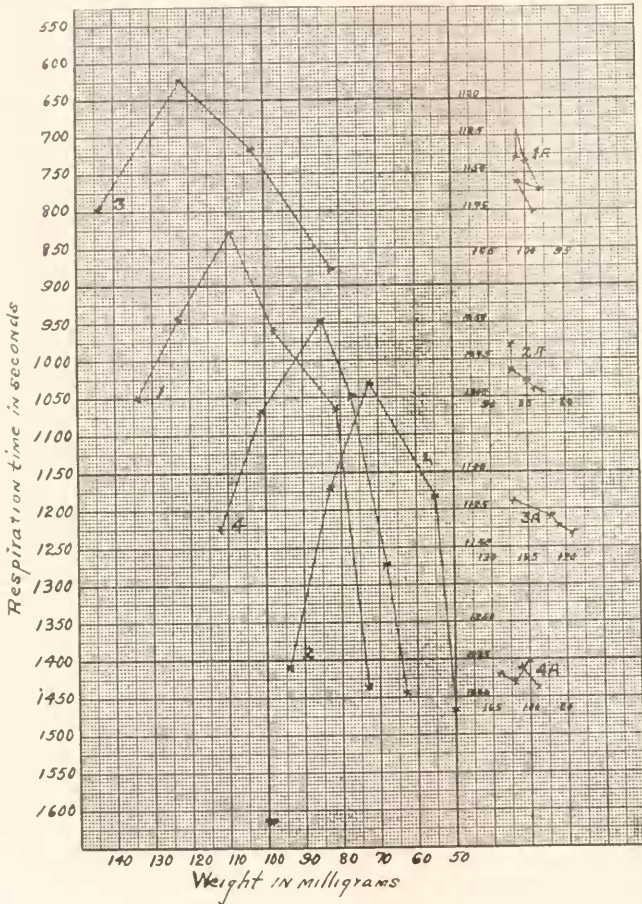readings became constant.

FIG. 4. The effect of desiccation on the carbon dioxide production of the slug.
Control curves indicated by letter (A) and enlarged to twice the scale of experi-
mental curves. Total time of each experiment about 8 hours.

The rate of carbon dioxide production increased with desic-
cation until there was a loss of water equivalent to from 20 to 30
per cent of the body weight when the rate fell below normal and

continued to decrease with further loss of water. The time
between the first and last determinations was too short to permit
the starvation factor to enter into the results. The controls lost
some water in the duration of a series, a period of about 8 hours.
One control lost 6.29 per cent. of the body weight, the greatest
loss of any control, while the experimental animal of the same
series lost 43.05 per cent. of the body weight. The respiration
rates of the controls seemed to be little, if at all, affected by the
slight losses of water.

Salamander, Plethodon glutinosus Green.—The salamanders
gave up water very quickly. Results show that 10 per cent. of
their body weight was lost in 15 minutes of desiccation. The
greatest loss of the four individuals experimented upon was 42.92
per cent. of the body weight, distributed over a period of 8 hours.
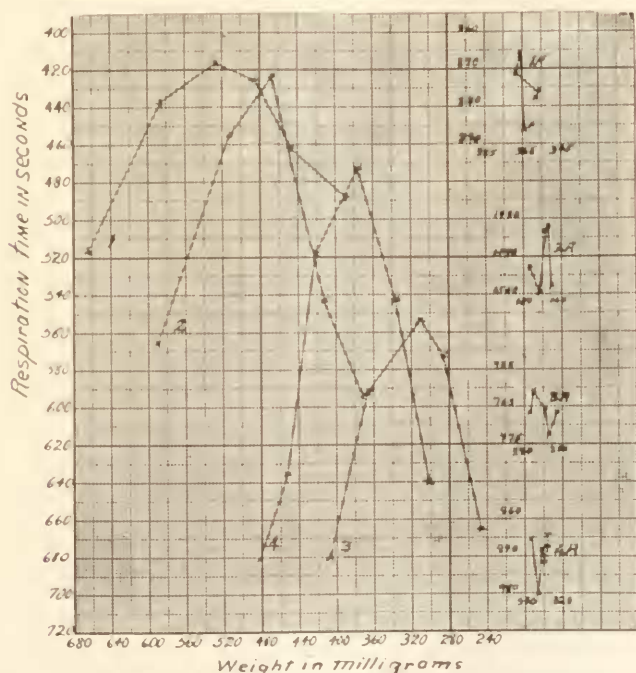

Fig. 5. The effect of desiccation on the carbon dioxide production of the
salamander. Control curves indicated by letter (A); numbers correspond to the
experimental curves. Control curves twice experimental scale.

This individual lived 6 hours after the last desiccation. Hall
(1922) desiccated *Ambystoma punctatum* to 47 per cent. of their

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body weight without loss of vitality. The salamanders proved easy to handle, oriented themselves negatively to light in the darker region of the animal chamber and remained quiet. When placed in the desiccator for the first time, the animals were quite active for 5 minutes, never longer. Carbon dioxide determinations prior to losses of 20 to 25 per cent. in body weight showed a rapid increase while continued desiccation resulted in a decrease in carbon dioxide output. The control animals lost very little water, not enough to appreciably affect the rate of carbon dioxide production.

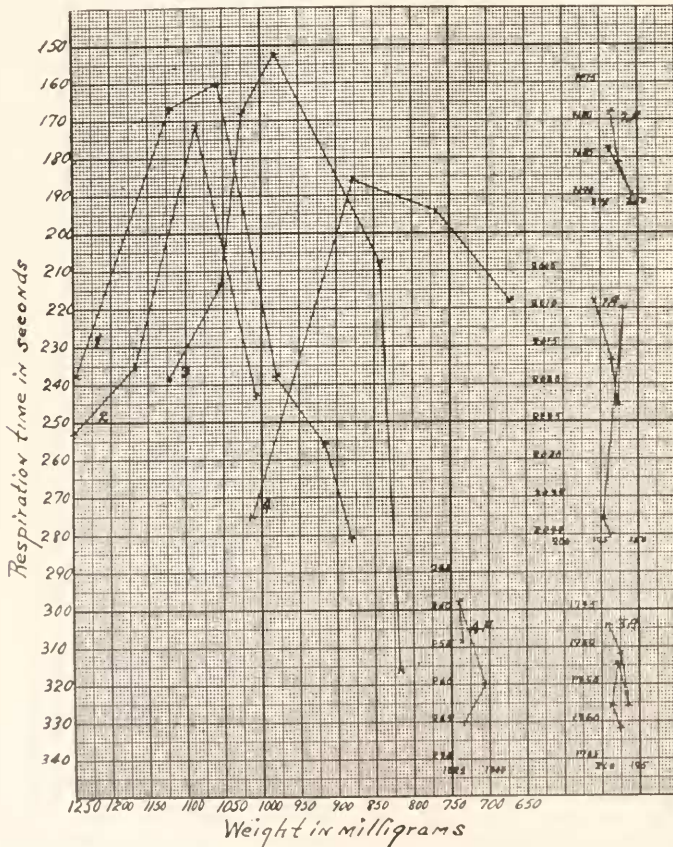*Frog, Acris gryllus Le Conte.*--Adult frogs of this species which

FIG. 6. The effect of desiccation on the carbon dioxide production of the frog. Points (x) on control curves (indicated by letter A) correspond to determinations on experimental curves. Control curves twice experimental scale.

had been kept in the laboratory for several days were used. To quiet the animals proved rather difficult. A dark chamber with a small opening in the paper covering caused the animals to orient themselves towards the light. After being handled the respiratory rates of the animals returned to normal very slowly, that is, a number of consecutive readings showed a decrease in rate before they became constant. This suggested the possibility of oxygen depletion within the closed system. Krogh (1916) in summarizing the work of several authors concluded: "In cold blooded animals oxygen consumption is practically independent of the oxygen pressure down to about 2 per cent. pressure." Fresh air was admitted to the animal chambers without disturbing the animal, by the removal of the indicator tube. This had no effect upon the rate of carbon dioxide production, so it was assumed that the animal had obtained sufficient oxygen. The rate of carbon dioxide production in the desiccated frogs of the four series of experiments increased with loss in weight until the loss amounted to from 15 to 20 per cent. of their body weight. The rate diminished subsequently to the completion of the experiment. The total time of a single experiment was less than 14 hours and hardly permitted of starvation as a factor in the decrease in respiration rate. The control animals lost very little water, from 0.26 to 1.47 per cent. The respiratory rates of the control frogs were fairly constant throughout the work.

V. Discussion.

These investigations show that desiccation stimulates the physiological activity of certain animals and that the increase in irritability is followed by marked depression. While there is no literature on the effects of desiccation on the carbon dioxide output of animals, it is interesting to note that Bodine (1922) obtained somewhat similar results from the action of ether, xylol and acetone upon grasshoppers, and concluded that anesthetics have physiological effects other than on respiration. Shelford (1913), with behavior methods, found that animals subjected to air of low humidity were more active than normal ones, and if kept under such conditions, the period of activity was followed by a period of depression, and suggested that the increased irritability was probably due to the concentration of the blood and

tissue fluids. Since the present results are based on physiological determinations, they are hardly comparable with results from behavior studies, however, it seems reasonable to suppose that if this were the entire cause of the stimulation, the increased irritability would continue, with additional losses of water, to a point nearer the vital limit of desiccation than has been found in the present experiments. Also, Shelford refers to irritability as evidenced by the activity of the animals, while these experiments show that the increased metabolic activity continues long after the animals become inactive. To cite a special case, the meal worms were active for the first 6 to 10 hours in the desiccator but the respiratory rate increased for more than 60 hours.

Since meal worms with a water loss of less than 1 per cent. of the body weight gave an increase in respiratory rate of more than 10 per cent., increased concentration of body fluids could hardly be the principal factor in causing the increase and suggests that this is, in part, a function of the nervous system. The truth of this assumption is open to investigation and further work will be done along this line.

There is a rough correlation between the highest respiratory rates and the vital limits of desiccation. According to Hall (1922), the vital limit of the frog is 41 per cent. and that of the salamander 47 per cent. The highest respiratory rate of the frog, from an average of four experiments, corresponded to 13.66 per cent. loss of body weight and with the salamander to 21.87 per cent. loss of body weight. The xerophilous animals gave up water very slowly and in proportion to water losses were stimulated more than the hygrophilous animals.

The decrease in carbon dioxide elimination with further desiccation is even more difficult to explain. No literature on studies pertaining to either starvation or desiccation of the larvæ of *Tenebrio* and *Alypia* has been found, but Hill (1911) showed that the heat production of the frog decreased with starvation and Child (1919), Hyman (1919) and Allen (1919) who worked with *Planaria*, and Bodine (1921) with grasshoppers, all pointed out that starvation decreased the rate of carbon dioxide production. The results from the meal worm experiments indicate a stimulation from desiccation until the depression of food starvation became a greater factor. Particularly, since the respiratory

rates of both the experimental animals and controls decreased after 48 hours of desiccation without food, while the fed controls showed only slight variations. Water starvation undoubtedly increases the depression of food starvation, but this fails to explain the decrease in respiratory rates of animals not affected by starvation.

In work on pithed frogs, the author has observed that handling often produced reflex movements, but after the frogs had lost considerable water these reflex movements did not occur, and furthermore, were not induced when the sides of the body or feet were pinched. This suggests the possibility that after the nerves have lost a certain percentage of water, the passage of the nervous impulse is retarded or inhibited. If this be true, in part or whole, the highest respiratory rate should immediately precede the disappearance of reflex movements, but no data are available on this point. Durig (1901) found that the nerves of a frog transmitted more slowly and the latent period of the muscles increased with water losses from 9 to 30 per cent. of the body weight, so that the decrease in respiratory rate is what might be expected if this were true.

Until more data are available, it is difficult to suggest the causal factors, but the effect will probably be found to be in part caused by accumulation of waste products due to poor elimination and also to inadequate distribution of food and oxygen to the cells. It is also highly probable that the decrease in water content has a direct action in slowing down certain of the oxidative processes. Decreased metabolism from any or all of these causes would appear in the experiments as conducted.

VI. SUMMARY.

1. Desiccation is followed by marked changes in the physiological states of certain animals.

2. *Agriolimax campestris* Binney, *Plethodon glutinosus* Green, *Acris gryllus* Le Conte, and larvæ of *Tenebrio molitor* Linn show a definite, positive, correlation between desiccation and carbon dioxide production.

3. The above-named animals show a gradual increase in carbon dioxide production when desiccated to less than one half of the possible vital limit. Continued desiccation is followed by a

decrease in carbon dioxide production, which falls below normal before the vital limit is reached.

4. The larvæ of *Alypia octomaculata* Fabricius show a decrease in carbon dioxide production in all stages of desiccation.

5. Xerophilous animals give up water more slowly and in proportion to water losses are more stimulated by dry air than hygrophilous animals.

6. Water starvation increases the depression of metabolic activity caused by food starvation.

7. Extremely dry air is a protoplasmic irritant.

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